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Loss of Epidermal Homeostasis Underlies the Development of Squamous Cell Carcinoma

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Abstract

Squamous cell carcinoma (SCC) is one of the most common skin cancers. To develop targeted therapies for SCC, a comprehensive understanding of the disease through a systems approach is required. Here, we have collated and analyzed the literature on SCC and pathways that maintain skin homeostasis. Since, the loss of the Notch and the overactivation of the Wnt pathways in the epidermis cause SCC, we focused on these two pathways. We found that the two pathways are critical in maintaining epidermal homeostasis. Further, we found that the cancer stem cell (CSC) marker CD44 causes the transcription of SOX2, another CSC marker of SCC, activates the Wnt pathway, and blocks the Notch pathway. Similarly, the Wnt pathway causes the transcription of CD44 and SOX2 and blocks the Notch pathway. In this paper, we have discussed how the notch and the Wnt pathways affect epidermal homeostasis and the three CSCs (CD44, SOX2, and LGR6) affect the two pathways, linking the CSCs with epidermal homeostasis.

Keywords Squamous cell carcinoma · CD44 · SOX2 · LGR6 · Notch · Wnt · Epidermal homeostasis

Introduction

Nonmelanoma dermatologic cancers are among the most common cancers [1]. Squamous cell carcinoma represents around 20% of all nonmelanoma skin cancers [1]. In a study spanning 15 years, the greatest increase was found in the cutaneous head and neck squamous cell carcinoma among SCCs, suggesting that exposure to sunlight is the major factor responsible for skin SCC incidence [2]. The use of tobacco and alcohol, and exposure to viral infections, such as HPV, Epstein-Barr virus, and hepatitis C virus, are the other factors that increase the risk of SCC development [3, 4]. Failure to remove cancer at the primary site is one of the most important reasons for a patient's death [5].

Head and neck squamous cell carcinoma (HNSCC) progresses from cellular atypia to various stages of dysplasia to invasiveness, yet, in most patients, it is detected in the late stage [6]. In addition to the well-known oncogenes/tumor suppressors, such as p53, CDKN2A, PTEN, PIK3CA, and HRAS, the genes that are involved in the squamous differentiation, such as Notch1, IRF6, and p63, are dysregulated in HNSCC [3]. Further, the notch pathway is upregulated in SCC, correlating with the aggressiveness of the disease [7]. On the other hand, the higher Notch activity acts as a tumor suppressor in SCC [8]. Similarly, the Wnt pathway genes are also upregulated in the disease [9]. Moreover, there are crosstalks between the two pathways in SCC [10]. Interestingly, the two pathways maintain epidermal homeostasis [11].

While the treatment of cancer by blocking molecular targets increases the life span of patients, in many cases the disease relapses and worsens. Thus, a comprehensive understanding of tissue homeostasis is required for the better management of the treatment options. In this review, we elucidate the role of the Wnt and the Notch pathways in the context of the known cancer stem cells of SCC and epidermal homeostasis.

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Epidermal Stem Cells, their Differentiation, and Epidermal Homeostasis

The Quiescence of Skin Stem Cells, the Homeostasis of Skin, and the SCC Development

Hair follicle stem cells undergo cycles of quiescence and activation [12]. These cells can generate squamous tumors in the active phase but not in the quiescent phase, suggesting that the property of quiescence plays a dominant role in suppressing the causes of carcinogenesis [12]. In contrast, SCC develops from the stem cell niche while the proliferating transit-amplifying cells fail to respond to tumorigenic stimuli [13]. Further, based on the gene expression profile of human SCCs, it has been found that SCCs may form due to a profound change in the homeostatic machinery of the skin stem cells, eliminating their quiescence, rather than due to a simple expansion of the stem cells [14].

Role of Notch Signaling in Epidermal Differentiation

In the canonical Notch signaling pathway, upon ligand binding to the notch receptor, the extracellular domain of the receptor is cleaved by ADAM. Then, γ-secretase, activated by a complex of Presenilin (PS), Nicastrin, PEN-2, and APH-1, cleaves the intracellular domain of the receptor (NICD) (Fig. 1A) [11]. Subsequently, NICD translocates to the nucleus and recruits the notch activator complex composed of RBP-Jk, MAML, and p300. Interestingly, besides the activator complex, a repressor complex composed of RBP-Jk, SHARP, CtBP, and CtIP also forms. The activator and the repressor complexes together control the transcription of the notch target genes (Fig. 1A) [11].

The canonical notch signaling is important for the differentiation of the epidermal cells [15, 16]. In this context, the notch activator and repressor complexes together control the cyclin D1 expression [11], which regulates epidermal differentiation. A moderate expression of cyclin D1, controlled by the notch activator and the repressor complexes together along with the Wnt pathway, is required to balance the proliferation of epidermal stem cells and their differentiation (Fig. 7 in [11]), maintaining skin homeostasis.

Further, Notch signaling is critical for maintaining proper growth conditions in the hair follicle matrix (HFMC) by blocking TGFβ signaling [17, 18]. Thus, Notch signaling is important for the proliferation of the hair follicle cells thereby affecting their differentiation [17, 18]. In addition, Notch signaling is required for preventing the hair follicle bulge cells acquire the fate of the interfollicular epidermis (IFE) [17], affecting skin homeostasis.

In Normal Skin, the Wnt Pathway Regulates the Stemness of the Epidermal Cells

In the canonical Wnt pathway, in the absence of a Wnt ligand, β-catenin is ubiquitinated and degraded due to its phosphorylation, first, by CKIα and CKIε, and subsequently by GSK3β [11]. A multiprotein complex organized by the scaffold protein axin is required for the β-catenin phosphorylation and subsequent degradation [11]. On the other hand, in the presence of a Wnt ligand, GSK3 β is displaced from the scaffold by Disheveled (Dsh), preventing the degradation of β-catenin and causing its translocation to the nucleus, activating the Wnt target genes [11] (Fig. 1B).

Leucine-rich repeat-containing G-protein coupled receptor 6 (LGR6), an enhancer of the Wnt pathway, marks the stem cells of the interfollicular epidermis [19]. $LGR6^+$ stem cells are present in the isthmus region of the hair follicle [20]. Further, the Wnt target gene, Axin2, has been shown to mark the epidermal stem cells [21]. Thus, the Wnt pathway has been implicated in maintaining the stemness of the epidermal cells. The Axin2 cells produce both the Wnt signal, in an autocrine manner, and long-range Wnt inhibitors [21]. This interplay between the Wnt activator and Wnt inhibitor may be involved in epidermal differentiation [22]. Previously, we hypothesized that there are four types of epidermal cells in the basal layer of the epidermis in a dynamic equilibrium governed by stochasticity [22]. LGR6 + /Lrig1 + cells are the major stem cells of the epidermis while three other cell types: LGR6-/Lrig1- cells, LGR6-/Lrig1 + cells, and LGR6 + /Lrig1- cells, are the different types of the transit amplifying cells [22]. Our model explains the presence of quiescent stem cells (LGR6 + /Lrig1 +) in the basal layer of the epidermis and supports a 2-compartment (stem cells-transit amplifying cells) model while weakening the gradual stemness hypothesis of this layer. The model provides the composition of the basal layer and explains the presence of β1 and α6 integrins throughout this layer.

Three Kinds of Crosstalks between the Wnt and the Notch Pathways

In addition to forming an activator complex, the Notch pathway effector, RBP-Jk, forms a repressor complex involving SHARP, CtBP, and CtIP [23] (Fig. 2A). Similarly, β-catenin forms a Wnt repressor complex involving CtBP [24] (Fig. 2A). Thus, the activators of the notch and the Wnt pathway also form corresponding repressor complexes involving CtBP. Therefore, the common pool of CtBP may act as a switch between the two pathways (Fig. 2A). The notch repressor complex, involving CtBP, may activate the Wnt pathway by depleting the CtBP from the Wnt repressor complex. Thus, the notch repressor complex regulates the stem cell compartment

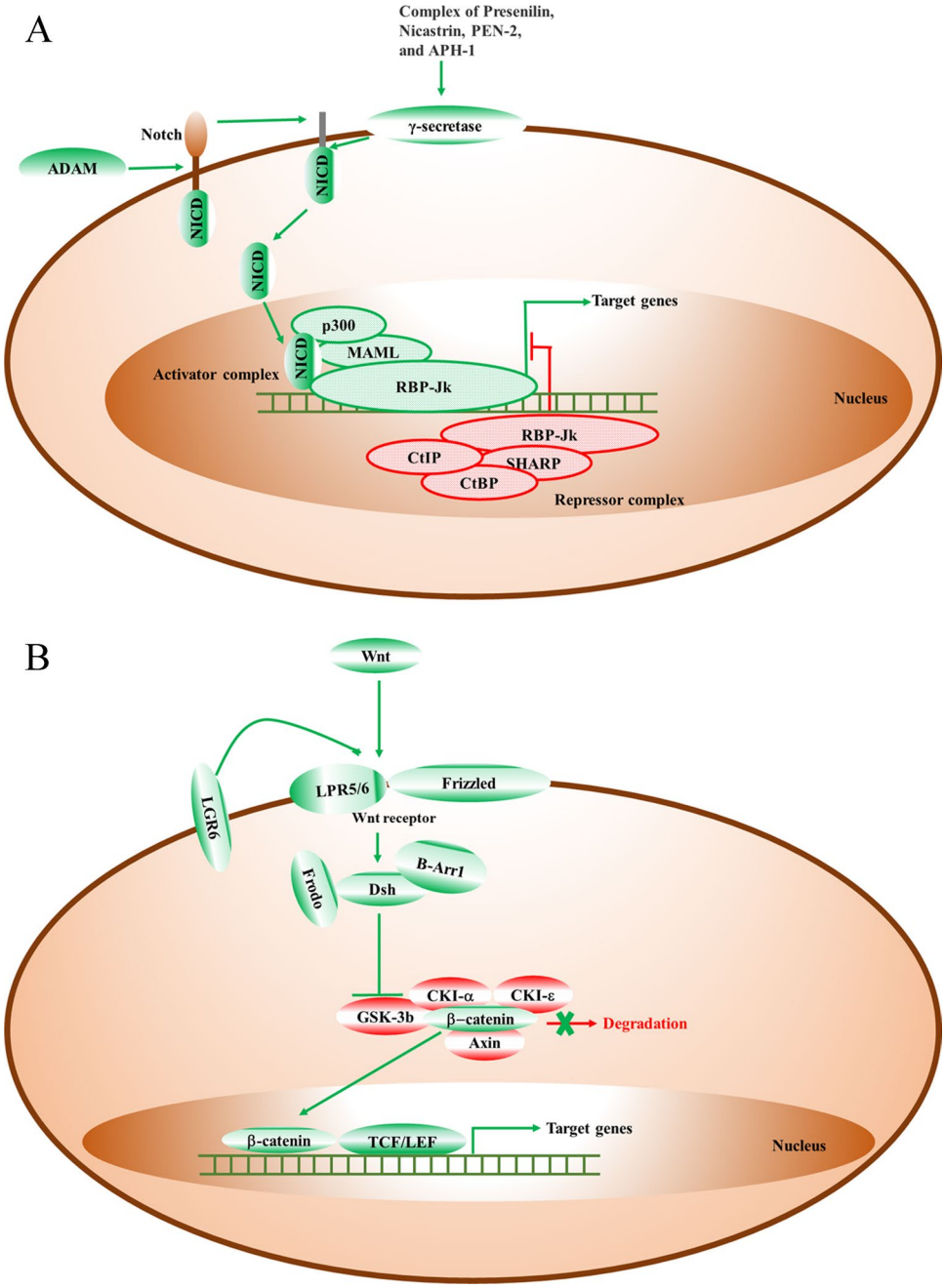
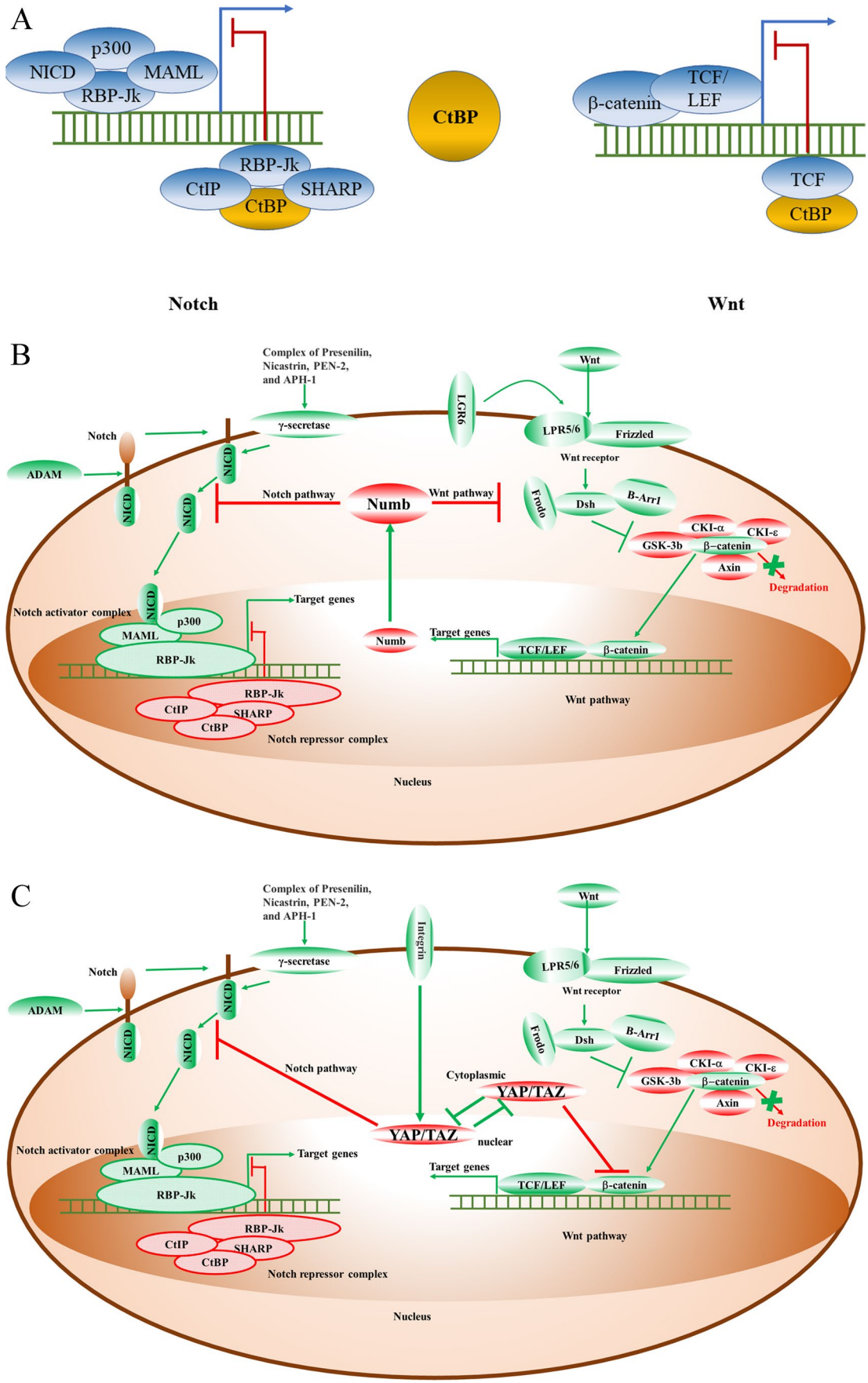


Fig. 1 (**A**) The Notch pathway. Upon ligand binding, the extracellular domain of the Notch receptor is cleaved by ADAM. Then, γ-secretase cleaves the intracellular domain, which translocates to the nucleus forming an activator complex involving RBP-Jk. Simultaneously, a notch repressor complex involving RBP-Jk and CtBP also forms. The activator and the repressor complexes together control the target genes (**B**) The Wnt pathway. In the absence of a Wnt ligand, β-catenin is degraded due to its phosphorylation by GSK-3β. Upon addition of a Wnt ligand, GSK-3β is displaced by Disheveled (Dsh) preventing β-catenin's degradation. Then, β-catenin translocates to the nucleus, activating the Wnt target genes

through the Wnt pathway and the differentiation of the epidermal cells through the notch pathway [11]. Therefore, the two pathways maintain epidermal homeostasis [11] and the common pool of the limiting protein CtBP creates the first crosstalk between the two pathways.

In addition, the persistent Wnt signaling can block the Notch signaling through Numb [25, 26] (Fig. 2B), regulating Numb through LEF/TCF [26, 27]. Similarly, Numb negatively regulates the Wnt pathway [28] (Fig. 2B). Since Numb is the common negative regulator of the two pathways, the common pool of Numb can cause a switch-like regulation where one pathway is ON while the other is OFF, creating the second cross-talk between the two pathways.

Further, the integrin-mediated signaling causes the nuclear localization of YAP/TAZ, the effector proteins of the Hippo signaling pathway [29], and regulates epidermal stemness by inhibiting the Notch pathway [30] (Fig. 2C), whereas in the absence of the integrin signaling YAP/TAZ is localized to the cytoplasm where they cause β-catenin degradation [31, 32] (Fig. 2C). Thus, YAP/TAZ has two mutually exclusive roles (Fig. 2C): (1) be involved in integrin signaling, localize to the nucleus and inhibit the Notch pathway, or (2) localize to the cytoplasm, be involved in β-catenin destruction complex and


A
p300
NICD
MAML
RBP-Jk
RBP-Jk
CtIP
SHARP
CtBP
CtBP
β-catenin
TCF/
LEF
TCF
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Notch
Wnt
B
Complex of Presenilin,
Nicastrin, PEN-2,
and APH-1
γ-secretase
Notch
ADAM
NICD
NICD
NICD
LGR6
Wnt
LPR5/6
Frizzled
Wnt receptor
Notch pathway
Numb
Wnt pathway
Frodo
Dsh
B-Arr1
CKI-α
CKI-ε
GSK-3b
β−catenin
Axin
Degradation
Notch activator complex
NICD
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and APH-1
Integrin
γ-secretase
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LPR5/6
Frizzled
Wnt receptor
Frodo
Dsh
B-Arr1
Notch pathway
Cytoplasmic
YAP/TAZ
CKI-α
CKI-ε
GSK-3b
β−catenin
Axin
Degradation
YAP/TAZ
nuclear
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NICD
p300
MAML
RBP-Jk
Target genes
Target genes
TCF/LEF
β-catenin
Wnt pathway
RBP-Jk
CtIP
SHARP
CtBP
Notch repressor complex
Nucleus

◂**Fig. 2** The antagonistic relationship between the Notch and the Wnt pathways. When the two pathways share a common negative regulator, one pathway turns ON while the other turns OFF due to the limiting amount of the shared protein. (**A**) The common represser CtBP is shared between the two pathways (**B**) The common negative regulator, Numb, is shared between the two pathways. (**C**) The common negative regulator YAP/TAZ is shared between the two pathways. The nuclear YAP/TAZ blocks the Notch pathway while the cytoplasmic YAP/TAZ blocks the Wnt pathway

inhibit the Wnt pathway. Therefore, the regulation of the Wnt and the notch pathways by YAP/TAZ is the third crosstalk between the two pathways. These three crosstalks between the two pathways create switches between them (Fig. 2A, B, C). These switches may turn on one pathway while blocking the activity of the other pathway. Thus, they may activate the Wnt pathway while inhibiting the notch signaling, increasing the susceptibility to develop SCC.

Cancer Stem Cells of SCC and the Roles of the Wnt and the Notch Pathways

$CD44^+$ CSCs of Squamous Cell Carcinoma

Recently, a $CD44^+$ population of tumor cells has been described to be the cancer stem cell in head and neck squamous cell carcinoma (HNSCC) [33]. These cells possess two important properties, self-renewal, and differentiation-resistance, of stem cells [33]. While $CD44^+$ cancer cells express cytokeratins 5 and 14, the basal cell markers, CD44- cancer cells express involucrin, a marker of keratinocyte differentiation [33]. Thus, CD44 regulates the stemness of keratinocytes in SCC. Further, $CD44^+$ cells express BMI1, which is involved in the self-renewal and tumorigenesis of the cells [33]. On the other hand, human ATP-binding cassette (ABC) transporter ABCB5, a drug efflux pump in oral squamous cell carcinoma (OSCC), may also be linked to cancer stem cells [34]. Interestingly, ABCB5 is expressed by $CD44^+$ OSCC cells [34]. Thus, through ABCB5, CD44 may cause drug resistance in cancer cells. Further, gene expression of the cells expressing a high level of CD44 and a low level of CD24 (CD44high-CD24low) shows a CSC-like profile in OSCC [35]. Furthermore, CD44high-CD24low cells show epithelial-to-mesenchymal transition (EMT) and drug resistance [35]. Thus, CD44 + cells possess stemness, self-renewal, differentiation-resistance, EMT, and drug-resistance properties of CSCs of SCC.

Crosstalk among CD44, YAP/TAZ, KLF4, and SOX2

Transcription factor KLF4, which is important for the barrier function of the skin [36] and terminal epidermal differentiation [37], has been found to negatively regulate the transcription of CD44 and cancer cell stemness [38]. KLF4 and CD44 expressions are mutually exclusive [38] (Fig. 3). On the other hand, YAP/TAZ maintain epidermal stemness by limiting the level of KLF4 [39] (Fig. 3). Thus, both CD44 and YAP/TAZ negatively regulate KLF4, inhibiting the epidermal differentiation and enhancing stemness property of the epidermis (Fig. 3). Therefore, CD44 and YAP/TAZ affect epidermal homeostasis negatively (Fig. 3) while KLF4 affects it favorably. Indeed, YAP/TAZ and CD44 are the essential proteins involved in the initiation of squamous cell carcinoma [40] (Fig. 3).

Further, TAZ transcriptionally activates SRY (sex determining region Y)-box 2 (SOX2) [41], another CSC marker of SCC. Furthermore, TAZ and SOX2 expression correlated with the worst survival in HNSCC [41]. Consistently, HNSCC CSC subpopulation CD44 + CD133 + is significantly enriched in both TAZ and SOX2 [41], suggesting that CD44 + CD133 + causes SOX2 expression through TAZ (Fig. 3). Moreover, CD44, YAP/TAZ, and SOX2 can be expressed by the same population of CSCs, enhancing stemness of the cells.

CD44: A Mesenchymal Stem Cell Marker

CD44, an adhesion molecule found on the cell surface of several cancers, interacts with extracellular matrix proteins, hyaluronan, and osteopontin, which are found in the microenvironment of cancers [42]. A glycovariant of CD44 is characteristically expressed by MSCs [43, 44]. Further, CD44 has been implicated in homing and migration of MSCs to the site of an injury [43, 45–47]. Furthermore, CD44 has been shown to orchestrate EMT in HNSCC, breast cancer, lung cancer, ovarian cancer, colon cancer, and melanoma [48–54], suggesting that CD44 confers EMT property to the CSCs of skin SCC.

Interaction of CD44 with the Wnt and the Notch Pathways

CD44, which is transcriptionally activated by the Wnt pathway, associates with the Wnt receptor LPR6, increasing the Wnt activity [55] and forming a positive feedback loop (Fig. 4). Thus, the higher Wnt activity may cause CD44 expression and vice versa. Interestingly, γ-secretase, which is involved in Notch pathway activation is also involved in the intramembranous cleavage of CD44, subsequent translocation of the active CD44 fragment (CD44-ICD) to the nucleus, and its transcriptional activity [56]. Thus, the involvement of the limiting component, γ-secretase, in CD44 signaling may deplete its pool required for the notch signaling activity, inhibiting the Notch pathway, and suggesting an antagonistic relationship between the CD44 and the notch pathways (Fig. 4).

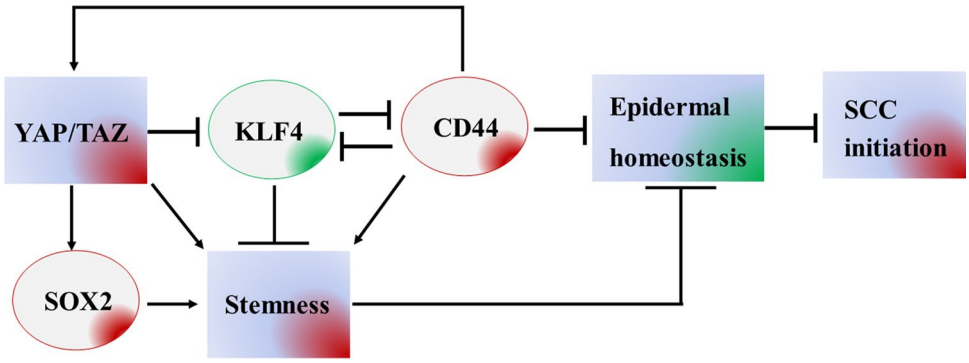

Fig. 3 Regulation of stemness by YAP/TAZ, CD44, SOX2, and KLF4, epidermal homeostasis, and SCC initiation. CD44 is positively regulated by YAP/TAZ through KLF4. On the other hand, YAP/TAZ is also positively regulated by CD44 while YAP/TAZ transcriptionally activates SOX2. Further, KLF4 and CD44 form a double-negative feedback loop. Through these regulations, CD44 may be involved in skin homeostasis and SCC initiation. Moreover, CD44, YAP/TAZ, and SOX2 positively regulate the stemness of epidermal cells while KLF4 regulates the stemness of these cells negatively. Thus, CD44, YAP/TAZ, and SOX2 regulate epidermal homeostasis negatively while KLF4 regulates it positively

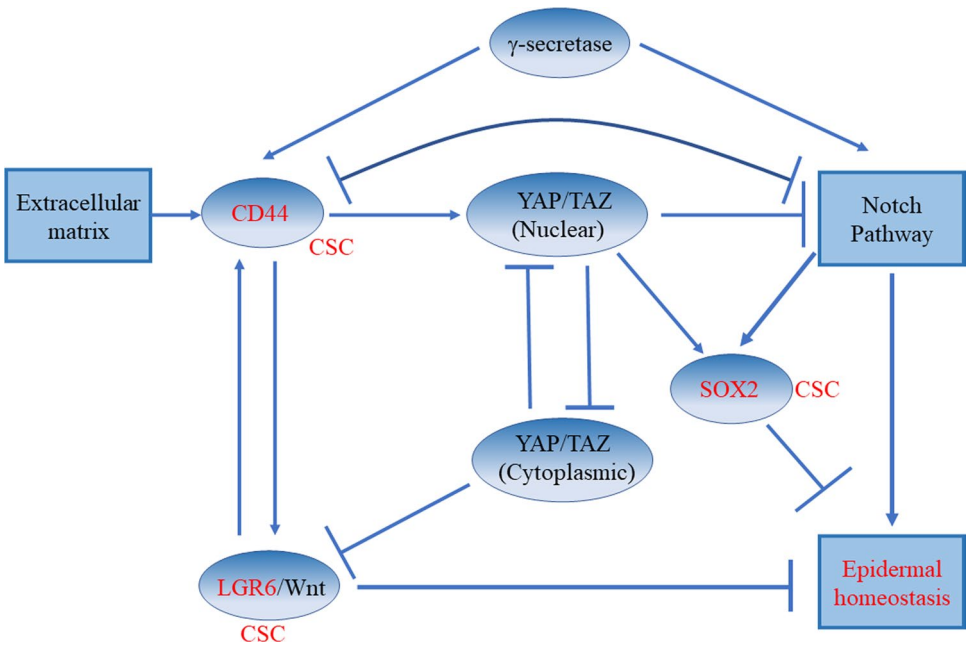

Fig. 4 Interaction between the three CSCs (CD44, SOX2, and LGR6) and the two pathways (Notch and Wnt). γ-secretase can be involved with either CD44 or the Notch pathway. Thus, when one of these two pathways is active, the other one is inactive. In addition, CD44 inhibits the Notch pathway through nuclear YAP/TAZ and activates the Wnt pathway through a positive feedback loop. On the other hand, the cytoplasmic YAP/TAZ blocks the Wnt pathway. Further, SOX2 is transcriptionally activated both by CD44 through the nuclear YAP/TAZ and by the Notch pathway. Between the two pathways, the notch pathway affects epidermal homeostasis favorably while the Wnt pathway affects it negatively. On the other hand, the three CSC markers (CD44, LGR6, and SOX2) negatively affect epidermal homeostasis

Similarly, the involvement of γ-secretase in the CD44 pathway, involving the extracellular matrix, may localize YAP/TAZ to the nucleus (Fig. 4), blocking the notch pathway while activating the Wnt pathway through the CD44-Wnt positive feedback loop (Fig. 4). Therefore, while the CD44 and the Wnt pathways activate each other through the positive feedback loop, they block the Notch pathway.

Conversely, the low-notch activity may activate CD44 by freeing γ-secretase. Further, since CD44 activates the Wnt receptor LPR6 [55], the low-notch activity may cause higher Wnt activity through CD44. Thus, interaction among the CD44, the notch, and the Wnt pathways may cause higher activities of the CD44 and the Wnt pathways while inhibiting the notch pathway, increasing the susceptibility to develop SCC.

Interestingly, a higher expression of Wnt pathway proteins, e.g., Wnt ligands Wnt-1, 10b, and Wnt receptor Fz-2, has been found in SCC [9]. Indeed, low-notch activity, high Wnt activity, and high CD44 expression can cause SCC. Further, an aberration in the CD44 expression can lead to an imbalance between the Wnt and the Notch pathways and vice versa, altering skin homeostasis, and this imbalance may be at the core of the mechanism responsible for the development of squamous cell carcinoma.

Role of Collagen Type VII in SCC Development in Recessive Dystrophic Epidermolysis Bullosa Patients, and the Relationship between CD44 and Collagen

Recessive dystrophic epidermolysis bullosa (RDEB), a blistering skin disorder frequently accompanied by the development of SCC, is caused by defects in type VII collagen [57]. RDEB keratinocytes lacking collagen VII are non-tumorigenic [57]. On the other hand, RDEB keratinocytes retaining a specific collagen VII fragment (noncollagenous domain NC1) generate Ras-driven tumors [57]. The fibronectin-like sequence in NC1 was required for tumorigenesis and caused tumor cell invasion [57]. Indeed, the expression of NC1 in RDEB patients increases their susceptibility to develop SCC [57].

Interestingly, collagen, critical for skin development and homeostasis, allows fitter, unstressed, and unaged epidermal stem cells to outcompete stressed keratinocytes, selecting unstressed epidermal stem cells to maintain epidermal homeostasis, modulating the skin aging [58]. Thus, defects in collagen may both affect epidermal development and cause RDEB-SCC.

Interestingly, dermal components also play a role in the development of SCC in RDEB patients [59]. In this context, RDEB-cancer-associated fibroblasts conferred adhesion and invasion property to keratinocytes in Wnt-5A dependent manner [59]. Further, RDEB dermal matrix has the property of the SCC-conditioned stroma with a role of TGF-b in the tumorigenesis [60]. Thus, drugs targeting the RDEB microenvironment may be a potential therapeutic option in RDEB-SCC patients [60].

Further, CD44 maintains an inflammatory response during wound healing through collagen [61]. In this context, CD44 causes collagenolysis and accumulation of fibrillar collagen during early wound healing [61]. In contrast, CD44 deletion leads to a higher fibrogenic response and decreases the tensile strength of the scar tissue [61]. Thus, CD44 regulates collagen deposition, its structure, and the strength of the extracellular matrix during wound healing. In summary, CD44 affects wound healing and inflammation through collagen whereas the defect in collagen in the extracellular matrix affects epidermal development and causes SCC in RDEB patients.

$SOX2^+$ CSCs of Squamous Cell Carcinoma

The transcription factor SRY (sex determining region Y)-box 2 (SOX2) has also been identified as a marker of the cancer stem cells in the skin SCC [62]. SOX2 is absent in the normal epidermis but is highly upregulated in the CSCs of the skin SCC [62]. Consistently, the deletion of SOX2 in mice led to a decreased tumor formation during chemically induced carcinogenesis [62]. To regulate the stemness properties of the cancer cells, SOX2 may act at several levels. For example, it may regulate stemness through CD133, MSI2, LIN 28, and HMGA2, proliferation through CCND2 and CDKN2A, chromatin regulation through SCMH1, metabolism through MG II, survival through ENPP1, and adhesion and invasion through PDPN and FLRT1 [62]. Further, by regulating CSCs, SOX2 perpetuates skin SCC initiation and progression [62].

Notch Causes SOX2 Expression, and Notch1 and SOX2 Define the Squamousness of Cancers

Notch signaling causes the expression of SOX2, activating the transcription factor Prox1 [63]. Further, Prox1 promotes lymph angiogenesis and is associated with many factors that define poor prognosis in oral SCC [64]. Furthermore, Prox1 is associated with advanced metastasis and the expression of hypoxia-induced factor 1α (HIF-1α) in esophageal squamous cell carcinoma [65]. Thus, Notch-SOX2-Prox1-HIF1α form an axis, which may be responsible for the poor prognosis of SCCs.

SOX2 and SALL4, another stem cell marker, were found to be upregulated along with many genes of the Notch pathway in esophageal SCC and correlated with the invasion and lymph node metastasis of cancer [66]. Further, since the notch pathway causes the expression of SOX2, and Notch1 and SOX2 expressions are altered in SCC of many origins correlating with poor prognosis, they are among the proteins that define the squamousness of cancers [67]. The notch pathway may be upregulated in certain cells of SCC to suppress tumorigenesis, however, if SCC develops despite the higher notch activity, the tumor acquires additional features, e.g., SOX2 expression, increasing aggressiveness of the disease.

CD44 and SOX2 Generate the Hybrid EMT State of SCC, Increasing the Aggressiveness of the Disease

SOX2, which is not expressed by normal skin epithelium, is required for cutaneous SCC growth through Nrp1/Vegf signaling [68]. Interestingly, in many SCCs, SOX2 has been shown to cause EMT [69, 70]. Consistently, the loss of function of FAT1, a protocadherin, causes a hybrid EMT state in SCCs by promoting an epithelial state through SOX2 and a mesenchymal state through CD44 [71]. Thus, both CD44

and SOX2 may be involved in the aggressiveness of SCCs by causing a hybrid EMT state of SCC cells.

LGR6$^+$ CSC of Squamous Cell Carcinoma

Leucine-rich repeat-containing G-protein coupled receptor 6 (LGR6), an enhancer of the Wnt pathway, but not LGR5 has also been found to be a cancer stem cell marker in the skin SCC [72]. Surprisingly, the loss of LGR6 predisposes mice to SCC development [72]. This apparent contradiction may be due to a compensatory upregulation of LGR5 in the absence of LGR6 [72], causing the higher Wnt activity. Thus, the higher Wnt activity may cause skin SCC by disturbing skin homeostasis.

LGR6 Expression Causes EMT, Increases Stemness, and Correlates with Prognosis in Squamous Cell Carcinoma

LGR6 is expressed in esophageal squamous cell carcinoma. Further, it is negatively related to the differentiation stage of the cells and correlated with the prognosis of the patients [73]. Furthermore, HPV + /LGR6 + patients are more likely to die from oropharyngeal SCC than the HPV + /LGR6- patients [74]. Thus, LGR6 expression correlates with a poor prognosis of SCC.

In addition, increased LGR6 expression was found in the patients of HNSCC specifically at the epithelial-stromal junctions, and correlated with more advanced disease [75], suggesting a correlation between the EMT, the LGR6 expression, and the stemness of SCC cells. Moreover, LGR6 expression has been found in the low differentiated oral SCC at the front edge of the invasion and is associated with tumor dormancy [76]. Thus, LGR6 has a role both in EMT and dormancy of SCC.

Consistently, EMT marker Twist1 has an important role in the development of UVB radiation-induced SCC and the expression of stem cell markers Lrig1 and LGR6 [77]. On the other hand, Twist1 is a Wnt target gene [78] (Fig. 5). Thus, there is a positive feedback loop (Fig. 5) between EMT marker Twist1 and the expression of stem cell markers Lrig1 and LGR6, connecting stemness with EMT in SCC development. Further, Lrig1, a stem cell marker like LGR6, may cause stem cell quiescence [79]. Therefore, LGR6 may cause EMT through Twist1 and tumor dormancy through Lrig1 (Fig. 5), conferring both EMT and stemness properties to SCC cells.

Inhibition of the Notch Signaling or Overactivation of Wnt Signaling May Cause SCC

Interestingly, blocking Notch signaling in the epidermis of mice causes the development of cutaneous SCC with the increased accumulation of β-catenin and cyclin D1, a Wnt target gene, in the nucleus [80], suggesting a link between the Notch and the Wnt pathways in SCC development. Further, concomitant overexpression of MAML1, a Notch signaling activator, and PYGO2, a Wnt pathway protein, was found in esophageal SCC patients, correlating with the tumor size and invasion [10]. Furthermore, in esophageal SCC, proinflammatory cytokine IL23 increased the CD133 cells and activated Wnt and Notch signaling pathways thereby attaining radioresistance [81], implicating both pathways together in the esophageal SCC.

On the other hand, many infrequent mutations in other genes in HNSCC converge on the inactivation of the Notch signaling, making Notch signaling inactivation one of the most prominent factors in developing HNSCC [82]. Since, LGR6, an enhancer of the Wnt signaling, has been identified as a CSC marker of SCC [72], we conclude that ablation of the Notch signaling, causing upregulation of the Wnt signaling, or independent overactivation of Wnt signaling may cause SCC. Further, higher expression of certain notch pathway effectors in SCC suggests that components of the Notch pathway are naturally upregulated during the development of SCC to suppress tumorigenesis. However, if SCC develops despite the higher-notch activity, the disease may be more aggressive.

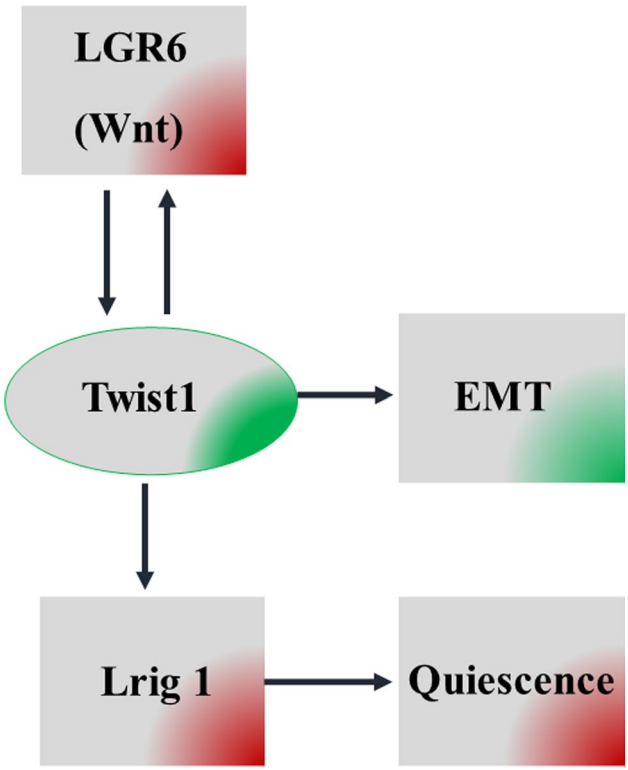

Fig. 5 LGR6 regulates the EMT and the quiescence of SCC cells through Twist1. LGR6 transcriptionally activates Twist1, causing EMT. Twist1 activates Lrig1, causing tumor dormancy

Perspective: Interaction Among the CSCs, the Wnt, and the Notch Pathways in SCC Development

Since the Wnt signaling causes expansion of the epidermal stem cells compartment, it affects the epidermal homeostasis negatively. On the other hand, since the notch pathway causes the differentiation of the epidermal stem cells, it affects epidermal homeostasis favorably. Below, we describe three scenarios in which the Wnt pathway is overactivated while the notch pathway is inhibited and the three CSC markers are expressed, causing SCC development.

In the first scenario, since γ-secretase is shared between the CD44 and the notch pathways, the expression of CD44 sequesters γ-secretase, deactivating the notch pathway (Fig. 4). CD44 also blocks the notch pathway through nuclear translocation of YAP/TAZ (Fig. 4). Furthermore, nuclear YAP/TAZ causes SOX2 expression (Fig. 4). In addition, CD44 and Wnt pathways mutually activate each other through the positive feedback loop between them (Fig. 4) Thus in this scenario, LGR6 causes the Wnt activation while the Wnt and the CD44 pathways mutually upregulate each other. On the other hand, Wnt and CD44 inhibit the notch pathway and cause SOX2 expression through the nuclear YAP/TAZ (Fig. 4). Thus, the three CSCs are expressed while the epidermal homeostasis is negatively affected.

In the second scenario, CD44 expression can activate the Wnt pathway through the positive feedback loop, cause SOX2 expression through YAP/TAZ, block the notch pathway through the sequestration of γ-secretase, and thus, disturb the epidermal homeostasis (Fig. 4).

Similarly, as a third alternative, the activation of YAP/TAZ leads to its nuclear translocation and blocks the notch pathway (Fig. 4), releasing γ-secretase to activate CD44 causing Wnt activity through the positive feedback loop, upsetting the epidermal homeostasis (Fig. 4). Further, the nuclear YAP/TAZ may cause the expression of SOX2 (Fig. 4).

Thus, in each of the three scenarios discussed above, the CSC markers of SCC are expressed while epidermal homeostasis is disturbed, causing the development of SCC.

Conclusion

SCC develops from stem cell niche while quiescence of stem cells confers protection against carcinogenesis. Further, the upsetting of tissue homeostasis may be the leading cause of carcinogenesis. A list of the target genes of the notch and the Wnt pathways (Table 1) shows that the two pathways are prominently involved in epidermal stemness, cell cycle, and epidermal differentiation, supporting their major role in maintaining epidermal homeostasis. CtBP is a common repressor of the two pathways. Similarly, YAP/TAZ and Numb are the common negative regulators of the two pathways. These negative regulators create a switch-like regulation of the two pathways, increasing the activity of one pathway while the other pathway is inhibited. In this context, the nuclear YAP/TAZ blocks the notch pathway while the cytoplasmic YAP/TAZ blocks the Wnt pathway. When nuclear YAP/TAZ is high, the cytoplasmic YAP/TAZ is low. Thus, the notch pathway is blocked while the Wnt pathway is activated. Therefore, YAP/TAZ may increase stemness and inhibit differentiation of the epidermal cells, negatively affecting epidermal homeostasis.

Table 1 Target genes of the notch and the Wnt pathways and their functions

Pathway	Target genes	Function
Notch	HES	Stemness, cancer metastasis
	CDK inhibitor (CDKN1A)	Cell cycle, cancer
	Cyclin D3	Cell cycle, cancer
	MYC	Epidermal differentiation
	HER2	Epidermal differentiation
Wnt	Cyclin D1	Cell cycle, cancer
	MYCN	Epidermal differentiation
	MMP7	Matrix remodelling, wound healing, Epidermal differentiation
	HNF1A	Drug resistance
	Axin2	Epidermal Stemness
	PPARG	Epidermal differentiation
	CD44	Skin barrier function, epidermal differentiation
	COX2	Abnormal epidermal differentiation

CD44, a mesenchymal stem cell marker, is also a SCC CSC marker. CD44 may cause the expression of SOX2, an epithelial state marker. Thus, CD44 and SOX2 together define a hybrid EMT state of SCC CSCs. Consistently, CD44 and SOX2 expressions are responsible for the worst prognosis of SCC patients.

Further, both the Notch pathway and the CD44 pathway are activated by γ-secretase. Thus, the expression of CD44 may inactivate the Notch pathway by depleting the common pool of γ-secretase. Furthermore, CD44 and Wnt pathways are linked through a positive feedback loop. Thus, CD44 expression may activate the Wnt pathway while inactivating the Notch pathway, either through the sequestration of γ-secretase or through nuclear translocation of YAP/TAZ (Fig. 4), increasing the stemness and blocking the differentiation of epidermal cells. Therefore, CD44 and the Wnt

pathway together affect epidermal homeostasis negatively and increase the stemness of SCC cells.

Like CD44, the Notch activity causes the expression of SOX2. The notch-SOX2 axis activates the hypoxia-induced factor, HIF1α, and is responsible for the poor prognosis of SCC patients. Further, Notch activity suppresses carcinogenesis however if SCC develops despite the higher notch activity, SCC shows a poor prognosis.

The Wnt pathway enhancer, LGR6, is another CSC marker of SCC. LGR6 causes EMT through Twist1 and confers the CSCs quiescence through Lrig1. Further, inhibition of the Notch pathway and overactivity of the Wnt pathway may cause SCC development by adversely affecting skin homeostasis.

Authors' Contributions Both authors performed the literature search and analysis. RS wrote the manuscript.

Data Availability All data are available in the manuscript and the supplementary information files.

Code Availability Not applicable.

Declarations

Ethics Approval Not applicable.

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Conflicts of Interest The authors declare that they have no conflict of interest.

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